Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

# PUBLIC HEALTH REPORTS

VOL. 33 FEBRUARY 8, 1918 No. 6

## THE QUALITY OF ANTIPNEUMOCOCCIC AND ANTIMENINGOCOCCIC SERUMS.

In order that those who use antipneumococcic and antimeningococcic serums shall be assured that such of these serums as are sold in interstate traffic are suitable for therapeutic purposes, each lot of these products made by the various manufacturers is tested at the Hygienic Laboratory of the United States Public Health Service prior to being placed on the market.

The quality of antipneumococcic serum is judged by its protective value against Type I pneumococci, using mice for test purposes.

Antimeningococcic serum is tested by agglutination and complement fixation tests, such as prove satisfactory by either test being passed.

## EFFECT OF FREEZING ON THE ORGANISMS OF TYPHOID FEVER AND DIPHTHERIA.[1]

By JOSEPH BOLTEN, Passed Assistant Surgeon, United States Public Health Service.

The occurrence during the summer of 1917 of an outbreak of diphtheria [2] and one of typhoid fever,[3] which seemed properly attributable to the ingestion of infected ice cream, constituted the reason for determining, under experimental conditions, how long the typhoid bacillus and the diphtheria bacterium would survive freezing, and whether freezing had any influence on the pathogenicity of the latter organism.

### Results with B. typhosus.

To determine the length of time typhoid organisms would live in cream that had been frozen solid, the following experiment was undertaken on October 4, 1917.

---

[1] From the Hygienic Laboratory.

[2] McCoy, Bolten, and Bernstein, Public Health Reports, vol. 32, No. 43, Oct. 26, 1917, pp. 1787–1804.

[3] Personal communication from Surgeon L. L. Lumsden.

Four metal containers were sterilized and partly filled with 50 cubic centimeters of pasteurized cream. These were then inoculated with 5 cubic centimeters of a 24-hour broth culture of *B. typhosus* (Rawling's), and immediately placed in a brine tank, where they were frozen within a short period. They were permitted to remain in this condition for 24 hours, when they were removed and partly melted by placing in a 37° C. water bath for 15 to 20 minutes. A loopful of material from each container was streaked over an Endo plate. The containers were then replaced in the brine tank and the Endo plates incubated at 37° C. for 24 hours. The next day the procedure was repeated. From the Endo plates, inoculated during the preceding day, typical typhoid colonies were fished into two nutrient broth tubes and on one Russell slant tube. These were incubated at 37° C. for 24 hours. The next day, macroscopic agglutination tests were performed. One drop of a high titer antityphoid serum, 1:2,000, was placed in one broth tube, while the second broth tube was used as a control. The tubes were incubated at 37° C. for half an hour. At the end of this period all the tubes to which serum had been added showed a heavy flocculent precipitate. The Russell slant tube showed a red butt, with no gas and unchanged slant.

This experiment was done daily from October 4 to November 1. After this date it was performed every second day, to November 28. During the first few days there was very little change in the number of colonies per plate, but after 10 days to two weeks there were about half the number, as compared with the original plates. At the end of the month, the number of colonies developed were very few, probably less than one-twentieth of the number of colonies that developed on the first few days.

From November 28 to December 17 the containers were left undisturbed in the brine tank On December 17 the experiment was resumed. The typhoid organisms in two containers were apparently dead, as no growth appeared on the Endo plates. The other two containers still gave a growth, one colony per loop from one and six colonies from the other. The colonies at this time were not the typical grayish translucent ones of typhoid. Instead they all presented a slightly reddish center. Subcultures in broth, however, were agglutinated satisfactorily and the appearance on the Russell medium plant was characteristic of the typhoid bacillus.

If we may assume that *B. typhosus*, under natural conditions, would resist freezing in cream as long as did this laboratory culture, there is no doubt that ice cream would be a very effective agent for distributing infection.

### Results with Bact. diphtheriæ.

In order to ascertain whether freezing would have any effect on viability and on the production of diphtheria toxin, 8 pure cultures of *Bact. diphtheriæ,* obtained from the epidemic referred to, were studied. These cultures were obtained from clinical cases of diphtheria and were isolated in pure cultures by plating on agar and fishing of colonies to Loeffler slants. The cultures were subjected to the following treatment:

(1) On September 26 two small Loeffler slant tubes were inoculated from each culture. These cultures were grown at 37° C. for 24 hours, after which they were each washed off with 10 cubic centimeters of 0.85 per cent salt solution and the suspension was placed in sterilized metal containers. These containers were then placed in a large wire basket and suspended in a brine tank, where in a short time they were frozen solid. They were allowed to remain in this tank in a frozen condition for 24 hours, when they were placed in a 37° C. water bath for 20 minutes, in order partly to melt the frozen solid. At the end of this period the tops were flamed and a loopful of material was inoculated on a Loeffler blood serum slant. The tubes were then incubated at 37° C. and the contents of the containers frozen again. The following day the process was repeated and the cultures of the inoculated tubes of the preceding day were examined. In all cases diphtheria bacilli were obtained. The freezing was continued for six days, and diphtheria organisms were recovered each day.

(2) On October 9 the experiment was repeated, with a slight variation. In this experiment, instead of using salt solution, 40 cubic centimeters of pasteurized cream were used. One 24-hour slant culture was washed in 5 cubic centimeters of salt solution and the 40 cubic centimeters of cream were inoculated. The cultures were frozen for four days, and then 2 per cent neutral peptone was inoculated with the frozen and the original (unfrozen) cultures.

The peptone-broth cultures were incubated at 37° C. for five days. On the fifth day 1 cubic centimeter of each of the broth cultures was injected subcutaneously into guinea pigs. Eight pigs were used for the frozen cultures and eight for the original unfrozen ones. All the pigs died within a period of three days, but without any significant difference in period of survival between those given frozen and those given the original cultures.

(3) On October 30 the experiment was again repeated. The amount of pasteurized cream used this time was 50 cubic centimeters; otherwise the details were the same as in the second experiment. The cultures were frozen for four days and then 2 per cent neutral peptone broth was inoculated with the frozen and original (unfrozen) cultures. The broth cultures were grown for four days at 37° C. and

were then used for the toxicity experiment. In the previous experiment we deemed the amount of culture used too large. In this experiment we used three dilutions, 0.5 cubic centimeter, 0.05 cubic centimeter, and 0.005 cubic centimeter, of each, frozen and unfrozen, culture. The injections were made subcutaneously.

The results of the test failed to show any difference between the toxin production of the frozen and of the unfrozen cultures.

An additional experiment of the same nature, in which the well-known diphtheria culture, Park No. 8, was frozen in the same manner for four days and then tested for toxin production, with a control of unfrozen, failed to show any difference between the two.